



## **Report of LibertyLink Rice Incidents**

### **Introduction**

This report summarizes the U.S. Department of Agriculture's (USDA) response to the low-level presence of two regulated lines of genetically engineered (GE) rice—LLRICE601 and LLRICE604—found in U.S. commercial rice. It is important to note that both GE rice lines have the same protein, which has been safely used in other deregulated products for more than 10 years. USDA's Animal and Plant Health Inspection Service (APHIS) initiated an investigation on August 1, 2006, after Bayer CropScience reported that regulated genetic material LLRICE601 had been detected in the long-grain rice variety Cheniere. The investigation was expanded on February 16, 2007, to include the discovery of regulated genetic material, later identified as LLRICE604, in the long-grain rice variety Clearfield 131 (CL131). APHIS has now completed the investigation.

APHIS' Investigative and Enforcement Services (IES), in coordination with USDA's Office of the Inspector General (OIG), investigated the events described in this report. The investigation was a multi-agency effort at both the Federal and State level. APHIS' Biotechnology Regulatory Services (BRS) program provided expertise in reviewing evidence, obtaining records, and assisting with subject interviews. USDA's Grain Inspection, Packers and Stockyards Administration (GIPSA) and Agricultural Marketing Service (AMS) provided molecular services that validated molecular identification tests and also provided molecular identification tests on rice seed, flour, and tissue samples collected. APHIS' Plant Protection and Quarantine program and GIPSA assisted in the collection of seed samples using standard techniques. USDA's Economic Research Service provided information regarding rice production and trade in the United States. Several State departments of agriculture and public research centers were helpful in providing samples and information for the investigation.

USDA devoted considerable resources to the investigation to ensure that it was conducted in a thorough and extensive manner. The investigation involved more than 8,500 staff hours gathering information across 11 States and Puerto Rico and site visits to more than 45 locations in 25 counties in 6 States. USDA officials tested 396 samples from 57 rice varieties that had been harvested between 2002 and 2006. Investigators were able to determine that the presence of LLRICE601 was limited to the long-grain rice variety of Cheniere and that the presence of LLRICE604 was limited to the long-grain variety CL131. No short- or medium-grain rice varieties tested positive for either LLRICE601 or LLRICE604. Investigators had hoped to identify how each GE rice line entered the commercial rice supply, but the exact mechanism for introduction could not be determined in either instance. However, direct cross-pollination probably was not a factor for LLRICE604's entry point into CL131.

Based on the findings of the investigation, APHIS will not be pursuing enforcement action against Bayer CropScience, the company that developed and field tested LLRICE601 and LLRICE604. Given the lack of available information and evidence,

APHIS was unable to make any definitive determinations that could have resulted in enforcement action. APHIS recognized at the start of the investigation that it faced a difficult task given that the field tests for these GE lines were conducted between 1998 and 2001. In addition, during the investigation, it was discovered that some records that might have been pertinent had not been maintained and were not available. Nevertheless, APHIS officials made every effort to learn as much as they could about the events that resulted in the unauthorized release of LLRICE601 and LLRICE604.

To protect the integrity of an investigation, it is APHIS' policy not to disclose information regarding an investigation until it is complete. However, as part of APHIS' commitment to transparency, certain information was released during this investigation to help farmers make decisions for upcoming planting seasons and to keep trading partners informed.

### **APHIS' Role in the Regulation of Biotechnology**

Under a coordinated regulatory framework, APHIS, the U.S. Food and Drug Administration (FDA), and the U.S. Environmental Protection Agency (EPA) share responsibility for regulating biotechnology products to ensure that the development, testing, and use of the products of biotechnology occur in a manner that is safe for plant and animal health, human health, and the environment. APHIS, through its BRS program, enforces the Plant Protection Act (PPA) with respect to biotechnology, regulating the importation, interstate movement, and field testing of GE organisms that might pose a risk to plant health. APHIS is committed to ensuring that this technology moves forward safely through its rigorous regulatory system.

On March 27, 2007, APHIS clarified its existing policy regarding the low-level presence (LLP) of regulated GE plant material in commercial seeds and grain. LLP is the mixing—at extremely low levels—of genes and gene products from unintended plant sources. This can occur with both conventionally bred plants as well as biotechnology-derived plants. These occurrences can result from natural processes such as the movement of seeds or pollen or human-mediated processes associated with field testing, plant breeding, or seed production. BRS continually examines confinement measures, including isolation distances, to ensure that they are adequate; however, these measures still might not prevent 100 percent of LLP occurrences.

When LLP incidents occur, APHIS' policy is to respond with actions appropriate to the level of risk, as determined by a scientific assessment and warranted by the facts in each case. APHIS' course of action when it announced that trace amounts of regulated GE rice had been found in commercial rice was consistent with its LLP policy. APHIS' first priority was to assess the safety of the GE rice. APHIS reviewed scientific data and information about the GE rice and determined that the GE rice poses no identifiable concerns related to agriculture or the environment.

APHIS will initiate an inquiry whenever regulated material is mixed with commercial seeds or grain to assess any risk, evaluate the circumstances surrounding the release, and determine whether remedial and/or enforcement actions may be appropriate.

### **Background on LibertyLink Rice**

Bayer CropScience developed LibertyLink lines of rice to allow the company's Liberty herbicide (glufosinate) to be sprayed on weeds without killing the rice plants. USDA has approved—and FDA has completed its consultation process for—two LibertyLink lines similar to LLRICE601, LLRICE06 and LLRICE62. However, they are not in commercial production. Federal authorities have concluded that LibertyLink rice poses no threat to food safety, human health, or the environment, and after thorough safety evaluations, APHIS extended deregulation to include LLRICE601 in November 2006.

These lines were produced by inserting the bar gene (35SBar), which encodes the enzyme phosphinothricin N-acetyltransferase (PAT). PAT provides resistance to the herbicide glufosinate, has a long history of safe use, and is present in many deregulated products. It has undergone repeated and thorough scientific evaluation and is used in food and feed, cultivation and breeding in the United States and many other countries. FDA has evaluated the PAT protein for safety on a number of occasions and has concluded that the presence of rice from the LLRICE600 series at low levels in food and feed would pose no safety concerns. APHIS has previously deregulated GE, herbicide-tolerant products such as corn, canola, and soybean that contain the PAT protein.

To facilitate the investigation, GIPSA verified two analytical methods that Bayer CropScience provided to detect LLRICE. Both tests are real-time polymerase chain reaction (PCR) methods. One detects the 35SBar DNA sequence found in LLRICE, and the other detects the DNA sequence specific to the LLRICE601 trait.

### **Initial Discoveries Pertaining to LLRICE601**

On July 31, 2006, Bayer CropScience orally informed BRS and FDA of the possible LLP of regulated GE rice in U.S. commodity rice. Preliminary information provided to USDA indicated that one long-grain rice variety, Cheniere, may have contained the 35SBar construct, although it was unknown at that time whether other varieties also could have contained the gene.

APHIS participated in discussions with FDA and EPA to consider food safety issues. BRS began to examine the possible source of the LLRICE601 by gathering information from Bayer CropScience on the exact identity of the genetic material. BRS also reviewed testing protocols provided by Bayer CropScience and reviewed all documents on rice field trials conducted by the company and its two predecessors, AgrEvo and Aventis. At the same time, BRS began to examine the planting history of the rice variety Cheniere.

### **Investigation of LLRICE601**

On August 1, 2006, IES initiated an investigation, and on August 21, 2006, USDA expanded the investigation to include OIG. The objective of the investigation was to determine the specific identity of the gene, the manner in which the LLRICE601 made its way into commercial rice, and whether any USDA regulations were violated.

IES invested 2,090 hours of investigative work in the first phase of this effort, which involved 15 IES investigators, 1 IES enforcement specialist, 1 IES field supervisor, and 3 IES intelligence analysts. In addition, three OIG agents were assigned to this effort. In 11 States—Arkansas, Colorado, Iowa, Louisiana, Mississippi, Missouri, North Carolina, Pennsylvania, Tennessee, Texas, and Virginia—as well as Puerto Rico, investigators conducted interviews and reviewed documents to determine if all parties involved had provided information to USDA within required timeframes of discovery. Evidence was provided showing that parties had notified USDA as soon as they had verified the presence of LLRICE601 in rice.

USDA cast a broad net to determine which varieties of rice in the United States may have contained LLRICE601. Seed materials representing 90 percent of rice seed from top breeding facilities in the United States were selected using recent seed certification production records. Initially, small-, medium-, and long-grain varieties were all sampled for testing. From these sources, 233 samples were taken of 57 varieties of rice from the main breeding centers in Arkansas (65), California (44), Louisiana (82), Missouri (12), Mississippi (5), and Texas (25). Because rice seed is not normally held for more than 2 years, the oldest samples that could be obtained were from 2002.

It is important to note that, in rice breeding, typical seed development begins with the planting of the “head row,” which produces seed for the breeder rice. Breeder seed is then used to produce foundation seed, which is grown by licensed plant breeders. Foundation seed can be used to produce either registered or certified seed, and it is typically a 6- to 7-year process from the harvest of the “head row” to the availability of certified seed for commercial use.

Of the seed material that was sampled, only 2003 Cheniere foundation rice seed samples tested positive for LLRICE601. All seven of the 2003 Cheniere rice seed samples tested positive, and all of these were derived or originated from Louisiana State University (LSU). None of the Cheniere rice seed samples for 2004, 2005, or 2006 tested positive for LLRICE601. Lineage was confirmed between the 2003 Cheniere foundation rice seed and both the 2005 Cheniere certified seed lots and 2006 commodity seed that originally tested positive for 35SBar.

Because the LLRICE601 trait was originally inserted in the variety Cocodrie, it was important to determine how the Cocodrie variety rice may have mixed with the variety Cheniere during its development. Investigators looked at all locations where LLRICE601 had been grown, visiting 44 locations in 25 counties in 6 States. They also interviewed 22 cooperators to determine the locations at which any rice seed production, particularly Cheniere variety development, coincided with Cocodrie LLRICE601 research.

Investigators determined that Cocodrie LLRICE601 and Cheniere were grown at the same location and at the same time at the Rice Research Center North Farm in Crowley, Louisiana, in 1999, 2000, and 2001 under a Bayer CropScience contract. The varieties were separated during those three years by distances of 210 feet, 3,000 feet, and 165 feet respectively. Cheniere was never planted on a location that had been previously occupied by Cocodrie containing LLRICE601, according to the records provided. Affidavits obtained verified that equipment cleaning had been accomplished by the parties involved at the Rice Research Center North Farm in Crowley, Louisiana, for all planting, harvesting, and cleaning operations during this period. Detailed maps were reconstructed from breeding records because maps had not been developed for each planting season at these sites. In addition, some records at LSU were not available while others records were never taken. As a result, all procedures employed at the site to assure confinement could not be verified.

Representative samples of the variety Cheniere from 1999-2002 could not be obtained because the breeder seed for the variety had not been maintained from this early stage of development. Because rice seed for the period 1999-2002 was no longer available, the exact mechanism for incursion of the LLRICE601 gene into the Cheniere variety, such as gene flow or mechanical mixture, was not determined.

### **Description of the LLRICE604 Incident**

In response to the LLRICE601 incident, the USA Rice Federation implemented an action plan to ensure that U.S. rice was free of GE material. The plan established a standard seed-testing protocol for the detection of the presence of the LibertyLink trait. In addition, the Arkansas State Plant Board notified BRS that up to 30 percent of the samples of CL131—a long-grain variety of rice developed by LSU that was to be sold as certified rice seed in the spring of 2006—had tested positive for the 35SBar gene. Based on this discovery, BRS subsequently initiated a second phase of the investigation on February 16, 2007.

The second phase focused on determining the specific identity of the genetic material, which was subsequently identified as LLRICE604, and how it was introduced into CL131. IES invested more than 500 hours in investigative work in this second phase of the investigation. APHIS officials involved in this phase included six IES investigators, one IES enforcement specialist, one IES field supervisor, and one IES intelligence analyst. In six States—Arkansas, Colorado, Louisiana, North Carolina, Tennessee, and Texas—as well as Puerto Rico, investigators conducted interviews and reviewed documentation.

On March 5, 2007, APHIS issued emergency action notifications (EANs) to alert processors and farmers that they should not further distribute or plant CL131 rice seed until the unidentified genetic material could be identified. As a result of early action by APHIS, only three acres of CL131 were planted, and APHIS provided the single affected producer with crop destruction information.

On March 26, 2007, after USDA conducted testing to identify the genetic material, AMS and GIPSA confirmed that the unidentified genetic material was LLRICE604. Investigators then initiated an effort to identify and trace back those lots of CL131 that were positive for LLRICE604. Determining the circumstances by which the genetic material LLRICE604, originally inserted into the variety Cocodrie, was introduced in CL131 required an analysis of the planting history of field trials containing both rice varieties, as well as sampling and testing CL131 seed lots from all phases in the seed development process.

Based on the fact that the identified material was LLRICE604, and based on FDA's previous food safety evaluations of the PAT protein, the possible presence of minute levels of LLRICE604 in CL131 posed no concern, and the rice was safe to process. Since LLRICE604 remained a regulated article, producers were not permitted to plant any CL131 that was being held under the EAN. APHIS did not prevent the movement of CL131 rice or seed from previous years, although that seed may not be planted.

In February 2007, the samples of varieties collected from LSU during the original LLRICE601 investigation were retested by USDA for 35SBar and LLRICE62. USDA collected additional samples of CL131 in Arkansas, Louisiana, and Texas beginning on March 3, 2007; testing for the LLRICE604 gene started at the end of March. Of the CL131 seed sampled, ultimately only one lot of seed tested positive for both 35SBar and LLRICE604. The seed lot of CL131, numbered EGF5G90, grown and distributed by Garrett Farms of Danbury, Texas, is the lot that has tested positive for LLRICE604 as both registered seed (2005) and certified seed (2006). Evidence acquired during the investigation did not indicate that the variety Cocodrie containing LLRICE604 was ever grown at the Danbury, Texas location, and other samples of rice collected by USDA at the location did not test positive for LLRICE604.

The foundation seed lot (2004, LSU) that was the parent to this registered seed did not test positive for LLRICE604; however, the LSU head row CL131 seed sample from 2004 tested positive for 35SBar and LLRICE62. Although material derived directly from LSU was testing negative for LLRICE604, it tested positive for the LLRICE62 trait. Investigators recognized that there was a chance that the levels of the LLRICE604 gene in these samples were so low that they may not be detectable from the samples that were obtained.

Based on the above results, investigators analyzed the history of all field trials containing both rice lines. Investigators determined that CL131 was developed at the LSU Rice Research Station between 1998 and 2001 and was subsequently released for commercial sale. The variety Cocodrie containing LLRICE604 was developed by Bayer CropScience (formerly AgroEvo) and was tested at various locations, including the LSU Rice Research Station North Farm in Crowley, Louisiana, between 1998 and 2000.

Records and affidavits obtained during the investigation showed that the development of these two varieties did not overlap in location at any time. Records provided also showed that CL131 was never planted on the same field plot as Cocodrie containing the

LLRICE604 line, so that volunteers were not likely a cause of mixture. Volunteers are plants that grow spontaneously from seed left by a previous crop. Because the development of these two varieties did not overlap in location and time, the most likely entry point for LLRICE604 into CL131 was through a means other than direct cross-pollination.

The prevalence of LLRICE62 in samples tested by HorizonAg and Producers Rice Mill corroborated observations made by the Arkansas State Plant Board. Based on that finding, as well as the occurrence in LSU samples tested by USDA, the investigation examined the possibility that a variety containing LLRICE62 and LLRICE604 may have mixed with CL131.

Because LLRICE604 was not detected in representative samples of breeding lines at LSU, the exact time period and means of incursion of the LLRICE604 gene into the CL131 variety was not determined.

### **Additional Information from the Investigation**

During the course of the investigation, IES discovered seven instances in which field trials were planted or not terminated during the period specified by APHIS requirements. However, these instances occurred between 2000 and 2002 and are no longer within the 5-year statute of limitations. More importantly, however, these infractions did not contribute to the LLP of LLRICE601 and LLRICE604 in U.S. commercial rice.

### **USDA's Continuing Commitment to the Safe Development of GE Organisms**

APHIS remains dedicated to fulfilling its role as part of the Federal framework for the regulation of biotechnology. Since 1987, when the first field trial for GE plants under APHIS regulations was approved, the Agency has effectively overseen approximately 13,500 field tests and has issued more than 70 determinations of nonregulated status for GE crops in the United States. These field tests were conducted at more than 79,000 sites. None of the products that have been deregulated have been reported to significantly impact the environment in a negative way. Rare occurrences involving the LLP of GE material in commercial seed or grain must be considered in light of USDA's long record of success in biotechnology regulation. USDA remains confident that its regulation of biotechnology is effective.

Nevertheless, biotechnology is a rapidly advancing science, and USDA understands that it must keep pace with this new technology by continually enhancing its biotechnology regulations. One of USDA's most significant biotechnology initiatives is a programmatic review of its regulatory framework. On July 12, 2007, USDA published a draft environmental impact statement (EIS) that evaluates potential revisions to its existing regulations regarding the importation, interstate movement, and environmental release of GE organisms. APHIS published a notice in the *Federal Register* on July 17, 2007, to announce the availability of the draft EIS and to seek public comments until September 11, 2007.

The draft EIS outlines several key areas APHIS is evaluating, including expanding its regulatory scope through additional provisions in the PPA, using a tiered permitting system based on potential environmental risk, and implementing a process for continued oversight of crops that do not meet the criteria for deregulation. USDA will consider possible future changes to the regulations based on the information in the draft EIS, public comments received, and the latest scientific information available.